Cancer Cell
Previews

Bioinformatic Mining of Gene Expression Datasets Identifies ETV1 as a Critical Regulator of Oncogenesis in Gastrointestinal Stromal Tumors

Brian P. Rubin[1,*]
[1]Departments of Anatomic Pathology and Molecular Genetics, Cleveland Clinic, Lerner Research Institute and Taussig Cancer Center, Cleveland, OH 44195, USA
*Correspondence: rubinb2@ccf.org
DOI 10.1016/j.ccr.2010.10.036

KIT mutations are the hallmark of gastrointestinal stromal tumors (GISTs). In a paper recently published in *Nature,* Sawyers and colleagues demonstrate that ETV1 collaborates with oncogenic KIT to initiate a GIST-specific transcriptional program. These results radically alter our view of GIST oncogenesis and have important implications for diagnosis and therapy.

Sarcomas are a diverse group of malignant neoplasms that are unified by the fact that each diagnostic entity displays mesenchymal differentiation. Although they are rare, many sarcoma subtypes exhibit simple karyotypes that underlie comparatively simple oncogenetic mechanisms that make them accessible to molecular dissection (Rubin et al., 2009). Hence, sarcomas have contributed disproportionately to what is known about mechanisms of oncogenesis versus what has been learned from more common tumors.

Gastrointestinal stromal tumors (GISTs) are the most common type of mesenchymal tumor of the gastrointestinal tract (Rubin et al., 2007). The genetic hallmarks of GISTs are constitutively activating *KIT* or *PDGFRA*, which encodes platelet-derived growth factor receptor alpha (PDGFRα), mutations. These mutations are present in approximately 85% and 7% of cases, respectively. Inhibition of KIT and PDGFRα with molecularly targeted inhibitors such as imatinib mesylate (Gleevec; Novartis Pharmaceuticals) stabilizes metastatic or recurrent GISTs in approximately 80% of patients; GISTs are therefore a paradigm for oncogene addiction and targeted therapy. Although imatinib is able to control GISTs clinically, complete tumor regression is seen in less than 3% of patients, necessitating lifelong therapy. Furthermore, approximately 50% of imatinib-treated GIST patients develop acquired imatinib resistance within 2 years of the initiation of therapy. Thus, although KIT inhibition has led to unparalleled therapeutic success in GIST treatment, GIST researchers realize that KIT inhibition alone is unlikely to cure GISTs.

It is interesting that the discovery of *KIT* mutations in GISTs, undeniably the most important discovery in the history of GIST research, came from a researcher outside of the field; this researcher, Dr. Yukihiko Kitamura, was mainly focused on the role of KIT in mast cells and allergic diseases (Hirota et al., 1998). The discovery followed publications from other laboratories demonstrating that loss-of-function *Kit* mutations led to loss of interstitial cells of Cajal (ICCs), a phenotype that had gone unnoticed in *Kit* mutant mice for decades. ICCs are pacemaker cells in the gut wall and are responsible for peristalsis. On the basis of these data, Kitamura hypothesized that gain-of-function *KIT* mutations would result in ICC tumors. Not only did Kitamura and colleagues find *KIT* mutations in GISTs, but they also proposed that ICCs were the cells of origin of GISTs. It was a happy coincidence that imatinib, which had been developed to inhibit BCR-ABL kinase, also inhibited KIT. Only 39 months elapsed between Kitamura's publication describing *KIT* mutations in GISTs and the report of the first patient successfully treated with imatinib (Joensuu et al., 2001). Imatinib is now first-line therapy for the treatment of GISTs.

Now, approximately 12 years after the exciting discovery of *KIT* mutations in GISTs, Dr. Charles Sawyers, another relative outsider, has blindsided the field with an important observation that has changed the present view of GIST pathogenesis (Chi et al., 2010). It is even more impressive that the observation was made from data that had been generated in the labs of GIST researchers. While performing an in silico bioinformatics analysis of publicly available GIST gene expression datasets for highly expressed transcription factors, the authors discovered that *ETV1* (ETS variant 1), which encodes a member of the ETS family of transcription factors that have been identified as oncogenes in several other cancers, was highly expressed in GISTs but not in other sarcomas. They then demonstrated that members of an ICC subset known to give rise to GISTs and ICC hyperplasia in mouse models expressing constitutively activated Kit also strongly express Etv1. Moreover, loss of Etv1 in a mouse *Etv1*$^{-/-}$ knockout model results in complete absence of the same ICC subset whose members strongly express Etv1. These results highlight ETV1 as a lineage and survival marker in ICC and GISTs.

To identify genes that are controlled by ETV1 in GISTs, the authors performed gene expression analysis of GIST cell lines that had been depleted of *ETV1* through the use of *ETV1*-specific shRNAs. Analysis of genes that were downregulated by *ETV1* knockdown demonstrated a negative correlation with genes that are upregulated in GIST and the ICC subset expressing ETV1. This suggested to the authors that ETV1 unleashes an ICC- and GIST-specific gene program. Subsequent analysis by chromatin immunoprecipitation and deep sequencing showed a strong correlation between ETV1 promoter and enhancer binding

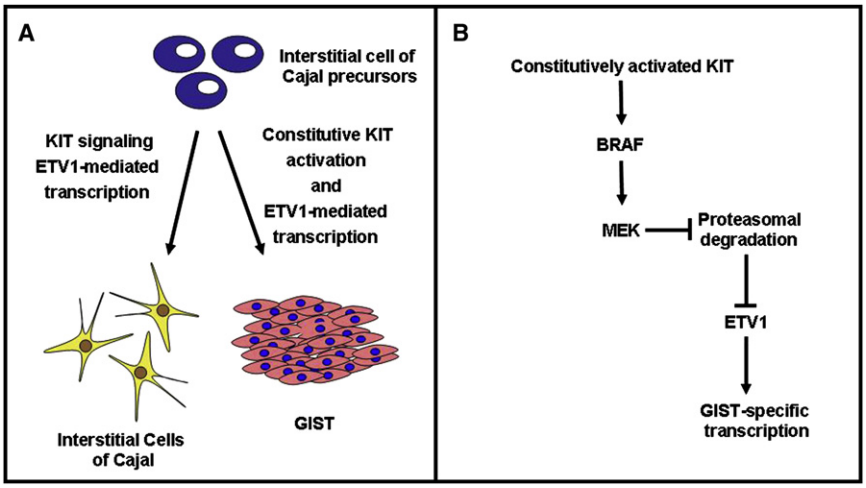

Figure 1. ETV1 Collaborates with KIT in GIST Formation
(A) ICC development is directed by both KIT and ETV1. When KIT is constitutively (oncogenically) activated, ETV1 transcription leads to GIST formation.
(B) At the molecular level, oncogenically activated KIT stimulates MAPK signaling, which prevents proteasomal degradation of ETV1 and leads to activation of a GIST-specific transcriptional program.

and subsequent ICC- and GIST-specific genes, further supporting the model that ETV1 is directly responsible for regulating ICC- and GIST-specific transcription.

From a therapeutic standpoint, it is exciting that the authors also demonstrated that shRNA-mediated knockdown of *ETV1* resulted in a reduction of GIST cell proliferation and decreased tumor volume in a mouse GIST xenograft model. Interestingly, tumors that did grow in this model escaped shRNA-mediated *ETV1* silencing, indicating that *ETV1* expression is critically important for GIST tumorigenesis. This result and the requirement for KIT and ETV1 in ICC development suggested that activated KIT and ETV1 might collaborate to transform ICC or ICC precursors into GISTs. In support of this hypothesis, there is evidence that constitutively activated KIT stabilizes ETV1 through MAPK signaling by inhibition of proteasomal degradation (Figure 1). Surprisingly, constitutively activated KIT and ETV1 were also shown to collaborate to transform NIH 3T3 cells. Together these results demonstrate that constitutively activated KIT and ETV1 are required for GIST tumorigenesis.

These studies have important implications for tumorigenesis in general and GISTs in particular. They highlight the importance of ETV1 as an oncogene by showing that its involvement extends beyond Ewing sarcoma, melanoma, and prostate cancer. The finding that ETV1 and constitutively activated KIT can collaborate to transform NIH/3T3 cells suggests that, in the proper context, ETV1 may be an oncogene in other cancers. Furthermore, ETV1 is rendered oncogenic in GISTs by a novel mechanism involving a combination of its high endogenous expression and stabilization by constitutive KIT activation. More significantly for GIST patients, ETV1 and MAP kinase signaling have been highlighted as new therapeutic targets, either alone or in combination with imatinib.

Members of a small subset of GISTs have neither *KIT* nor *PDGFRA* mutations but do contain the BRAF V600E mutation (Agaram et al., 2008). In light of the findings from Chi et al., it is interesting to speculate that ETV1 is stabilized in BRAF V600E mutant GISTs through KIT-independent MAPK signaling. Moreover, their results suggest that other mutations within the MAPK signaling pathway or even within ETV1 itself might be responsible for driving oncogenesis in GISTs that do not have *KIT* or *PDGFRA* mutations or in GISTs with acquired imatinib resistance; such GISTs initially respond to imatinib but develop imatinib resistance after a minimum of 6 months of therapy. Approximately 50% of the latter cases have intra-allelic *KIT* mutations that abrogate imatinib binding (Heinrich et al., 2006), but the mechanism of resistance in the remaining 50% is unknown. ETV1 also has the potential to be an excellent diagnostic marker for GISTs, especially for the approximately 5% of GISTs that are not immunoreactive for KIT. Finally, this work began with the mining of pre-existing datasets, supporting the argument that investments in bioinformatic infrastructure within individual labs as well as at the institutional, national, and international levels has the potential to fully exploit the immense amount of publicly available data that have been generated by the recent explosion of genome, transcriptome, and proteome projects.

ACKNOWLEDGMENTS

Work in B.P.R's laboratory is currently supported by generous grants from the Life Raft Group and the Scott Hamilton Cares Initiative.

REFERENCES

Agaram, N.P., Wong, G.C., Guo, T., Maki, R.G., Singer, S., Dematteo, R.P., Besmer, P., and Antonescu, C.R. (2008). Genes Chromosomes Cancer *47*, 853–859.

Chi, P., Chen, Y., Zhang, L., Guo, X., Wongvipat, J., Shamu, T., Fletcher, J.A., Dewell, S., Maki, R.G., Zheng, D., et al. (2010). Nature *467*, 849–853.

Hirota, S., Isozaki, K., Moriyama, Y., Hashimoto, K., Nishida, T., Ishiguro, S., Kawano, K., Hanada, M., Kurata, A., Takeda, M., et al. (1998). Science *279*, 577–580.

Heinrich, M.C., Corless, C.L., Blanke, C.D., Demetri, G.D., Joensuu, H., Roberts, P.J., Eisenberg, B.L., von Mehren, M., Fletcher, C.D., Sandau, K., et al. (2006). J. Clin. Oncol. *24*, 4764–4774.

Joensuu, H., Roberts, P.J., Sarlomo-Rikala, M., Andersson, L.C., Tervahartiala, P., Tuveson, D., Silberman, S., Capdeville, R., Dimitrijevic, S., Druker, B., and Demetri, G.D. (2001). N. Engl. J. Med. *344*, 1052–1056.

Rubin, B.P., Lazar, A.J.F., and Oliveira, A.M. (2009). Molecular pathology of bone and soft tissue tumors. In Cell and Tissue Based Molecular Pathology, R.R. Tubbs and M.H. Stoller, eds. (Philadelphia, PA: Churchill Livingstone Elsevier), pp. 325–359.

Rubin, B.P., Heinrich, M.C., and Corless, C.L. (2007). Lancet *369*, 1731–1741.